

Application No.: 09/271,584

2

Docket No.: 529642000200

AMENDMENTS**In the Specification**

Please insert the following new paragraph after the paragraph ending on line 17 of page 29:

--As described in table 4, "highly stringent conditions" are those that include a wash in 0.1xSSC, 0.1% SDS, at 60°-65° C. Exemplary conditions include initial hybridization in 6X SSC, 5X Denhardt's solution, 100 µg/ml fish sperm DNA, 0.1% SDS, at 55° C for sufficient time to permit hybridization (e.g., several hours to overnight), followed by washing two times for 15 min. each in 2X SSC, 0.1% SDS, at room temperature, and two times for 15 min. each in 0.1X SSC, 0.1% SDS, at 60° C, and preferably at 65° C, followed by autoradiography. The nucleic acid molecule is capable of hybridizing under highly stringent hybridization conditions when the hybridization mixture is washed at least one time in 0.1X SSC at 60° C, and preferably at 65° C.--

Please amend the paragraph beginning on line 2 of page 39 as follows:

The invention includes oligonucleotide probes made from the AtNHX sequences described in this application or other nucleotide sequences of the invention. The probes may be about 10 to 30 or 15 to 30 nucleotides in length and are preferably at least 30 or more nucleotides. A preferred probe is 5'-TTCTTCATATATCTTTTGCCACCC-3' SEQ ID NO:36 (coding for the amiloride binding domain) or at least about 10 or 15 nucleotides of this sequence. The invention also includes an oligonucleotide including at least 30 consecutive nucleotides of an AtNHX molecule in Figure 1 or 5 (or a partial sequence thereof). The probes are useful to identify nucleic acids encoding AtNHX, polypeptides and proteins other than those described in the application, as well as peptides, polypeptides, and proteins have Na⁺/H⁺ transporter activity and preferably functionally equivalent to AtNHX. The oligonucleotide probes are capable of hybridizing to one or more of the sequences shown in Figure 1 or 5 (or a partial sequence thereof) or the other sequences of the invention under low, moderate or high stringency hybridization conditions. A nucleotide sequence encoding a polypeptide of the invention may be isolated from other organisms by screening a library under low, moderate or high stringency hybridization conditions with a

sf-1557607

Application No.: 09/271,584

3

Docket No.: 529642000200

detectable probe (e.g. a labeled probe). The activity of the polypeptide encoded by the nucleotide sequence may be assessed by cloning and expression of the DNA. After the expression product is isolated, the polypeptide is assayed for Na⁺/H⁺ transporter activity as described in this application.

Please amend the paragraph beginning on line 9 of page 55 as follows:

The full-length cDNA of AtNHX1 was cloned by us from an Arabidopsis thaliana (Columbia) seedling cDNA library (38). The library was initially screened with an EST (GenBank # T75860; Figure 8(h)) obtained from the Arabidopsis Biological Resource Center (ABRC) that showed homology to Arabidopsis genomic sequence (A-TM021B04.4). The invention includes nucleic acid molecules of between about: 500-1000, 1000-1500 1500-1600, 1600-1700, 1700-2000 or 2000-2500 or greater than 2500 nucleotides including the EST sequence (or a sequence having at least about: 35, 35, 55, 65, 75, 85, 90, 95, 99, 99.5 sequence identity to the EST sequence or the polypeptide encoded by the EST sequence) and which encodes a polypeptide that extrudes monovalent (preferably potassium ions or lithium ions, most preferably sodium ions) out of the cytosol for preparation of transgenic plants and host cells, and in the other methods of the invention described below. These sequences are useful in the methods of the invention described above (for example as a probe, research uses, hybridization). The Arabidopsis genomic sequence predicted a polypeptide of 457 amino acids. Plaques that hybridized with the labeled EST probe were subjected to a secondary screen using the PCR product from the nested amplification of a region coding for the N-terminal portion of the predicted polypeptide. The forward primer, based on the predicted start codon of the polypeptide (Primer-NT), 5'-GCCATGTTGGATTCTCTAGTGTCG-3' SEQ ID NO:11 and the reverse primer, based on the stop codon predicted from the EST (Primer-CT), 5'-CCGAATTCTCAAAGCTTTTCTTCCACG-3' SEQ ID NO:12, were used to amplify a 1.7 kb product from the seedling library. This product was purified by agarose gel electrophoresis and used as the template for a second amplification using primer-NT and a reverse primer (primer-C) based on the genomic sequence, 5'-CGGAATTCACAGAAAAACACAGTGAGGAT-3' SEQ ID NO:13. The resulting 900 bp fragment served as the template for the probe used in the secondary screen. The pure plaques obtained in the secondary screen were tested by PCR using the primer-NT, primer-CT combination. Three of the plaques, from which a 1.7 kb product was amplified, were selected

sf-1557607

Application No.: 09/271,584

4

Docket No.: 529642000200

for excision of the phagemid. Single colonies containing the excised phagemid were grown in liquid culture. Aliquots of each of these cultures were used as templates for the PCR amplification of the region bound by the library plasmid to the 5' side of the clone (T3 promoter) and the reverse primer C. In one clone, a 1.2 kb fragment was amplified, which implied that the clone had an upstream untranslated region of approximately 300 bp. This clone was selected for complete sequencing.

Please amend the paragraph beginning on line 10 of page 56 as follows:

The full-length AtNHX2 cDNA was cloned from an *Arabidopsis thaliana* (Columbia) seedling cDNA library. PCR primers were designed for the amplification of the AtNHX2 sequence based on a BAC DNA sequence (MTE17) with a predicted amino acid sequence showing homology to AtNHX1. The forward primer (X6F), 5'-CCTCAGGTGATACCAATCTCA-3' SEQ ID NO:32 and the reverse primer (X6REV), 5'-GATCCAATGTAAACACCGGAG-3' SEQ ID NO:33 were used to amplify a 1.2 kb product from the seedling library by PCR. This product was purified by agarose gel electrophoresis and used as a probe in hybridization screening of the seedling cDNA library. Plaques that hybridized with the labeled probe were subjected to a secondary screen using the 1.2 kb PCR product as a probe. Pure plaques obtained in the secondary screen were tested by PCR using primer - X6F, primer - X6REV combination. Only one of the plaques had the 1.2 kb product amplified from it. This plaque was used for excision of the phagemid. This clone was used for complete sequencing.

Please amend the paragraph beginning on line 24 of page 56 as follows:

Full length AtNHX3 and AtNHX4 cDNAs were cloned by us from an *Arabidopsis thaliana* (Columbia) seedling cDNA libraries (CD4-15 and CD4-16; Arabidopsis Stock Center, Columbus, Ohio). PCR primers were designed for the amplification of a genomic sequence based on a BAC DNA sequence (F20D21) with a predicted amino acid sequence showing homology to both AtNHX1 and AtNHX2. The forward primer (NHX7F), 5'-TTCGTTCTCGGCCATGTCC-3' SEQ ID NO:34 and the reverse primer (NHX7REV), 5'-CGGAGAGACCAACACCTTCTGC-3' SEQ ID NO:35 were used to amplify a 2.2 kb product using *Arabidopsis thaliana* (Columbia) genomic DNA

sf-1557607

Application No.: 09/271,584

5

Docket No.: 529642000200

as a template. This product was purified by agarose gel electrophoresis and used as a probe in hybridization screening of the seedling cDNA libraries. Plaques that hybridized with the labeled probe were subjected to a secondary screen using the 2.2 kb PCR product as a probe. Pure plaques were used as templates for the PCR amplification of the region bound by the library plasmid using the T3 and T7 promoter sequences as primers. Two independent clones (insert sizes of 1.7 kb and 2.1kb) were selected for phagemid excision and complete sequencing.

sf-1557607